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# Optimization and validation of strategies for quantifying chromium species in soil based on speciated isotope dilution mass spectrometry with mass balance

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Strategies were designed to quantify hexavalent (Cr(VI)), soluble trivalent (Cr(III)) and insoluble chromium (Cr) species in soil by integrating existing methods of Cr(VI) and total Cr determination with speciated isotope dilution mass spectrometry (SIDMS, EPA Method 6800). Two different extraction methods that utilize a $NaOH–Na_2CO_3$ solution (EPA Method 3060A) and alkaline solution of ethylenediaminetetraacetic acid (EDTA) were used to extract Cr(VI) (along with soluble Cr(III) in the latter case). The extracted Cr was speciated by ion chromatography-inductively coupled plasma mass spectrometry (IC-ICP-MS), and the separated species were quantified using the mathematical relationships in SIDMS with simultaneous correction for their method-induced transformations. The Cr species that fell out as insoluble solid during extraction were determined by isotope dilution mass spectrometry (IDMS) after decomposing the extraction residues in a mixture of mineral acids according to EPA Method 3052. Several certified reference materials and a soil sample were analyzed using the proposed strategies. The measured mass fractions of Cr(VI) and total Cr in the reference materials statistically agreed with the certified values at 95% CL. The insoluble fraction of Cr accounted for 64–107% of the total Cr in the samples. The validity of the strategies was proved using mass balance by comparing the sum of the mass fractions of Cr(VI), soluble Cr(III) and insoluble Cr with the total Cr measured in the corresponding samples; the latter was determined by IDMS after decomposing the soils using EPA Method 3052 with single spiking.

## Introduction

Soil naturally contains a small amount of chromium (Cr) that comes from weathering of its bedrock.[1] The element also enters the terrestrial environment through anthropogenic activities such as dumping of industrial and municipal waste, dry atmospheric fallout from smelting, and combustion of coal and liquid fuels.[2] While Cr can exist in several oxidation states ranging from −(II) to +(VI), only the +(III) and +(VI) forms, which display contrasting behaviours, are prevalent in soil and other parts of the environment.[3] In soil, Cr(III) mainly exists as insoluble (hydr)oxides or adsorbs to humic acid and macromolecular clay compounds, whereas Cr(VI) occurs as anions ($CrO_4^{2-}$ or $HCrO_4^-$) that are mobile under most conditions.[4,5] Cr(III) is relatively innocuous and essential for the proper functioning of living organisms,[6] in contrast, Cr(VI) is toxic to both plants and animals being corrosive, acute tissue irritant and carcinogen.[7,8]

The speciation analysis of Cr has received increasing interest because of the opposing properties of the two prevalent species of the element. In soil, such analyses are mostly conducted based on the selective extraction of Cr(VI).[9] Attempts have not been made much to determine Cr(VI) along with Cr(III) (the latter mainly exists as insoluble Cr in soil)[4,5] possibly because of the extreme challenge in the simultaneous extraction of the two species and the less far toxicity of Cr(III). Although soil often contains much higher Cr(III) than Cr(VI),[10] the stability of the species can be affected by various factors that include soil pH, aeration, soil moisture content, UV light, microbial activities[1,11] and presence of oxidizing substances such as manganese (hydr) oxides.[1,5] Therefore, determination of Cr(VI) along with soluble Cr(III) and the insoluble fraction of Cr is beneficial to better understand the distribution of the Cr species and their long-term fate in accordance with the nature and composition of the soil. Furthermore, such comprehensive speciation analysis helps to validate the analytical results by mass balance comparison. Mass balance, where the sum of the measured concentrations of the species equals the total elemental

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content, is one of the definitive means of validating analytical results.

Accurate and precise determination of Cr(VI) and Cr(III) is precluded by the dynamic natures and variable stabilities of the species that can potentially cause their transformation and/or interconversion as a result of sample matrix complexity and external conditions imposed in the system during sample collection, storage, preparation and/or analysis.[9] This necessitates the use of methods that can account for the effect of such transformations of species. The speciated isotope dilution mass spectrometry (SIDMS) methodology, EPA Method 6800,[12] is uniquely capable to track and correct for errors originating from species-crossover as well as partial analyte recovery, analyte loss, signal suppression and instrument drift. The method involves spiking the sample with analogues of the target species enriched with different isotopes of their common central element at the very beginning of the sample preparation procedure. Samples are analyzed by a mass spectrometer (after the preparation steps), and analyte concentrations are calculated, with simultaneous correction of errors, by mathematical relationships that use isotopic ratios (not absolute intensity) and known constants without involving calibration curves. The fundamentals, mathematical algorithms and applications of SIDMS have been discussed in patents,[13,14] EPA Method 6800,[12] a book chapter[15] and several articles[16–20] published by the authors' research group.

The present study devised strategies to determine Cr(VI), soluble Cr(III) and insoluble Cr in soil by integrating existing methods of Cr(VI) and total Cr determination with EPA Method 6800. Among the various methods reported for Cr(VI) extraction from soil,[9,10,21] those employing hot alkaline solutions of $Na_2CO_3$–NaOH (EPA Method 3060A)[22] and ethylenediaminetetraacetic acid (EDTA)[23] were used. The use of hot alkaline solutions ensures complete dissolution of chromate compounds,[24–26] fresh $Cr(OH)_3$ and free Cr(III)[25] leaving other Cr(III) species such as $Cr_2O_3$ and aged $Cr(OH)_3$ in solid phase. Both alkaline extractions were carried out in a microwave after double-spiking the soil samples with isotopically labeled analogues of the Cr species, *i.e.* $^{53}$Cr(VI) and $^{50}$Cr(III). The extracts were analyzed by ion chromatography-inductively coupled plasma mass spectrometry (IC-ICP-MS) and the mass fractions of Cr(VI) and soluble Cr(III) were determined using the mathematical relationships in SIDMS. The insoluble fraction of Cr was determined after decomposing the extraction residues in a mixture of mineral acids following EPA Method 3052.[27] The digests were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) and the mass fraction of the insoluble Cr was determined by the isotope dilution mass spectrometry (IDMS) component of EPA Method 6800.[12] The proposed procedure was validated by analyzing several standard reference materials, and using mass balance by comparing the sum of the mass fractions of Cr(VI), soluble Cr(III) and insoluble Cr with the total Cr found in the corresponding soil sample; the latter was determined by IDMS after spiking the soil with $^{53}$Cr and decomposing it in acid. To the best of the author's knowledge, such a comprehensive determination of Cr species in soil based on SIDMS through systematic design of strategies and mass balance validation has not been reported.

# Experimental

## Instrumentation and software

Extraction and sample decomposition were performed using an Ethos EX microwave extraction system (Milestone). The instrument can process a total of forty one samples at a time, and it was equipped with temperature and pressure feedback control and magnetic stirring capability. Teflon microwave vessels were used for sample decomposition, and disposable polypropylene centrifuge tubes (50 mL, VWR) with perforated caps were used for extraction to avoid sample contamination by acid residuals from the Teflon vessels.

The ion chromatographic system was Metrohm 850 Professional IC (Metrohm). The system was metal-free (made from polyetherether ketone (PEEK)) and consisted of an auto-sampler (858 professional sample processor), a six-port sample injector, a pump, a column thermostat, and an eluent degasser. The optimum conditions of the chromatographic method are described in Table 1.

A model 7700x ICP-MS (Agilent Technologies) equipped with a micro-mist nebulizer, a quartz spray chamber, an octopole reaction system (ORS$^3$), and a quadrupole mass analyzer was used. The argon and helium were of ultra high purity grade (99.999%, Airgas). The instrument was tuned on the day of every analysis using an Agilent tuning solution that contained 1 μg $L^{-1}$ Li, Co, Y, Ce, and Tl in 2% $HNO_3$. Sensitivity (million counts-per-second, Mcps, per mg $L^{-1}$) of 40 ($^7$Li), 130 ($^{89}$Y) and 65 ($^{205}$Ti) or higher were achieved. The maximum oxide (CeO/Ce) and doubly charged ($Ce^{2+}$/Ce) ratios were 1.16% and 0.76%, respectively. For direct analysis by ICP-MS, samples were introduced using an auto-sampler (ASX-500 Series, Agilent Technologies), which is kept in an anti-contamination enclosure (ENC500, CETAC Technologies). For the IC-ICP-MS configuration, the IC column was connected to the ICP-MS nebulizer inlet using a switching valve. The ICP-MS sample line and the IC injection system were rinsed in a three-step procedure using 1% HCl, 1% $HNO_3$, and ultrapure water before the analysis of each sample. The operating conditions of the ICP-MS instrument are listed in Table 1.

Agilent MassHunter software (version G7201A A.01.01, Agilent Technologies) was used for ICP-MS data acquisition and chromatographic peak integration. Raw data were exported in Microsoft Excel compatible format to calculate isotope ratios. Cr-SPC™ software in the Cr speciation analysis Kit from Applied Isotope Technologies, Inc. was used to quantify the analytes by IDMS and SIDMS.

## Chemicals and standards

Ultra trace grade $HNO_3$ (69%) and hydrofluoric acid (HF, 48%), and tetrasodium salt of EDTA (>99%) from Fisher Scientific, NaOH (>97%), $Na_2CO_3$ (99.5%), $K_2HPO_4$ (98.0%) and $KH_2PO_4$ (99.0%) from BDH, anhydrous $MgCl_2$ (Aldrich), and ultrapure water (18.2 MΩ cm, Branstead NANOpure) were used. All the reagents were of analytical grade.

Stock standard solutions of natural abundant (Nat) and isotopically enriched Cr species, *i.e.* $^{Nat}$Cr(III) (10 μg $g^{-1}$),

Table 1 Optimum operating parameters of the IC and ICP-MS instruments

| **IC** | **Metrohm 850 Professional** |
|---|---|
| Column | Metrosep A Supp 4 anion exchange column (Metrohm), 250 mm long, 4.0 mm i.d., 9 μm particle size, 71 μmol $Cl^-$ capacity, working pH 3–12 |
| Mobile phase | 2 mmol $L^{-1}$ EDTA in ultrapure water, pH 10 adjusted using ammonium hydroxide |
| Elution mode | Isocratic |
| Flow rate | 0.8 mL $min^{-1}$ |
| Column temperature | Ambient |
| Injection volume | 100 μL |
| **ICP-MS** | **Agilent 7700** |
| RF power | 1550 W |
| RF matching | 1.8 V |
| Sampling depth | 8 mm |
| Plasma gas (Ar) flow | 15 L $min^{-1}$ |
| Carrier gas (Ar) flow | 0.95 L $min^{-1}$ |
| Makeup gas (Ar) flow | 0.15 L $min^{-1}$ |
| $ORS^3$ gas (He) flow | 4 mL $min^{-1}$ |
| Spray chamber temperature | 2 °C |
| Cones | Ni |
| Isotope monitored | $^{50}Cr$, $^{52}Cr$, $^{53}Cr$ |
| Data acquisition mode | Spectrum[a], time resolved analysis (TRA)[b] |
| Integration time per mass | 0.99[a] s, 0.1[b] s |
| Peak pattern | 3 points per mass[a] |
| Replicates per analysis | 4[a], 1[b] |

[a] ICP-MS. [b] IC-ICP-MS.

$^{Nat}Cr(VI)$ (10 μg $g^{-1}$), $^{50}Cr(III)$ (727 μg $g^{-1}$) and $^{53}Cr(VI)$ (96 μg $g^{-1}$), were provided in the Cr speciation analysis Kit by Applied Isotope Technologies, Inc. The $^{50}Cr(III)$ and $^{53}Cr(VI)$ standards were enriched with 97.64% $^{50}Cr$ and 93.06% $^{53}Cr$, respectively. Working solutions were prepared by diluting the stock solutions in water; all measurements were made by mass with 0.00001 g precision. All solutions were stored in Teflon bottles at 4 °C, away from ultraviolet lamp and sunlight.

## Soil samples

Five soil reference materials with certified or proposed certified mass fractions of Cr(VI) or total Cr, and one soil sample (soil 01) were analyzed in this study. The reference materials were CRM SQC012, hexavalent chromium in soil (Sigma-Aldrich RTC);[28] candidate SRM 2700, hexavalent chromium in contaminated soil, low level (NIST); SRM 2709a, San Joaquin soil (NIST) and SRM 2711a, Montana II soil (NIST). The certified Cr contents of the soil materials are given in Tables 2 and 3. Candidate SRM 2700 had two forms that were prepared by diluting another SRM (SRM 2701: hexavalent chromium in contaminated soil, high level) with high purity ground quartz and glass. The two 2700 candidate SRMs are referred in this study as 2700q and 2700g, respectively. SRM 2701 is very active matrix material made from soil contaminated with Cr ore processing residue, which is a waste left after ore extraction where Cr was purposefully converted from Cr(III) to Cr(VI) in a recovery process of soluble Cr(VI) before discard. This type of sample is unique and does not have the Cr chemistry typical of most contaminated soils.[29]

All the procedures pertaining to storage, sample preparation and analysis of the samples were carried out in a clean room equipped with a class-100 high efficiency particulate air filter hood.

## Sample decomposition for total Cr determination

The sample decomposition procedure was based on EPA Method 3052 (ref. 27) integrated with the IDMS component of EPA Method 6800.[12] A 0.2 g portion of a soil sample was weighed out into three microwave vessels and spiked with $^{53}Cr$ standard (quantified by mass) at expected analyte (total Cr)-to-spike ratio in the range 0.5–1.5; the optimum spike ratio for Cr ($^{52}Cr/^{50}Cr$ and $^{52}Cr/^{53}Cr$) spreads over two orders of magnitude (0.1–10).[20] Then, 9.0 mL of concentrated $HNO_3$ and 0.5 mL of HF were added into each spiked sample. After swirling the mixtures to ensure wetting and mixing, magnetic stirrers were put into each vessel. The vessels were sealed and irradiated in the microwave system at 180 °C for 10 min with appropriate ramp time (the ramp time varied between 15 to 30 min depending on the number of vessels loaded into the instrument). The sample digests were cooled to room temperature, diluted to 25.0 mL with ultrapure water and stored in a clean cold-room at 4 °C. Three method blanks were prepared.

## Extraction of Cr(VI) from the soil samples

For extraction using EPA Method 3060A,[22] 0.2 g of a soil sample was weighed out into three polypropylene centrifuge tubes and spiked with $^{53}Cr(VI)$ and $^{50}Cr(III)$ standards. Both standards were

 

Table 2 Mass fractions of total, hexavalent and insoluble Cr determined by EPA Methods 3060A and 3052 integrated with SIDMS, and percentages of method-induced interspecies conversions ($n = 12$, 95% CL)

| | Certified values, μg g$^{-1}$ | | SIDMS, μg g$^{-1}$ | | IDMS, μg g$^{-1}$ | | |
|---|---|---|---|---|---|---|---|
| Sample | Total Cr | Cr(VI) | Cr(VI)[b] | Conversion (%) Cr(III) to Cr(VI) | Insol. Cr[c] | Total Cr[d] | Sum of species[e] μg g$^{-1}$ |
| SQC012 | — | 56 ± 3.04 | 53.8 ± 0.8 | 1.6 ± 0.8 | 109 ± 6 | 159 ± 4 | 163 ± 7 |
| 2700q | — | 14.9 ± 1.2[a] | 14.5 ± 0.7 | 0.6 ± 0.03 | 346 ± 8 | 377 ± 9 | 361 ± 9 |
| 2700g | — | — | 13.6 ± 0.1 | 0.5 ± 0.03 | 684 ± 19 | 680 ± 14 | 698 ± 19 |
| 2709a | 130 ± 9 | — | 1.3 ± 0.2 | 2.0 ± 0.5 | 124 ± 4 | 122 ± 3 | 125 ± 4 |
| 2711a | 52.3 ± 2.9 | — | 0.8 ± 0.2 | 2.2 ± 0.6 | 53.5 ± 2.6 | 52.1 ± 2.1 | 54.3 ± 2.8 |
| Soil 01 | — | — | 1.0 ± 0.1 | 2.8 ± 0.2 | 58.3 ± 3.4 | 54.4 ± 2.1 | 59.3 ± 3.5 |

[a] Proposed certified value taken from ref. 23. [b] SIDMS values after correcting for interspecies conversions. [c] Insoluble Cr determined by IDMS after decomposing the extraction residues. [d] Total Cr determined by IDMS after decomposing the soil samples. [e] Sum of Cr(VI) and insoluble Cr.

Table 3 Mass fractions of total, hexavalent and insoluble Cr determined by alkaline extraction using EDTA solution and EPA Method 3052 integrated with SIDMS, and percentages of method-induced interspecies conversions ($n = 12$, 95% CL)

| | Certified values, μg g$^{-1}$ | | SIDMS[b], μg g$^{-1}$ | | | | IDMS, μg g$^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Conversion (%) | | | | |
| Sample | Total Cr | Cr(VI) | Cr(VI) | Cr(III)[c] | Cr(III) to (VI) | Cr(VI) to (III) | Insol. Cr[d] | Total Cr[e] | Sum of species[f], μg g$^{-1}$ |
| SQC012 | — | 56 ± 3.04 | 54.9 ± 1.4 | 2.0 ± 0.2 | 0.2 ± 0.09 | 6.2 ± 0.9 | 101 ± 7 | 159 ± 4 | 158 ± 2 |
| 2700q | — | 14.9 ± 1.2[a] | 13.5 ± 0.4 | 10.3 ± 0.5 | 0.4 ± 0.06 | 4.4 ± 0.9 | 343 ± 8 | 377 ± 9 | 366 ± 9 |
| 2700g | — | — | 10.1 ± 0.3 | 11.2 ± 0.9 | 0.2 ± 0.01 | 4.6 ± 1.2 | 644 ± 15 | 680 ± 14 | 665 ± 16 |
| 2709a | 130 ± 9 | — | 0.9 ± 0.1 | 3.2 ± 0.3 | 1.6 ± 0.3 | 5.2 ± 0.8 | 120 ± 5 | 122 ± 3 | 124 ± 5 |
| 2711a | 52.3 ± 2.9 | — | 0.6 ± 0.1 | 1.2 ± 0.2 | 0.9 ± 0.1 | 3.3 ± 1.1 | 48.1 ± 2.8 | 52.1 ± 2.1 | 49.9 ± 3.1 |
| Soil 01 | — | — | 0.7 ± 0.1 | 1.1 ± 0.2 | 1.8 ± 0.3 | 4.1 ± 0.9 | 50.9 ± 3.7 | 54.4 ± 2.1 | 52.3 ± 4.0 |

[a] Proposed certified value taken from ref. 23. [b] SIDMS values after correcting for interspecies conversions. [c] Soluble Cr(III) determined in the alkaline extract by SIDMS. [d] Insoluble Cr determined by IDMS after decomposing the extraction residues. [e] Total Cr determined by IDMS after decomposing the soil samples. [f] Sum of Cr(VI), soluble Cr(III) and insoluble Cr.

spiked at expected analyte-to-spike ratio of 0.5–1.5. Then, 12.0 mL of a solution containing 0.5 mol L$^{-1}$ NaOH and 0.28 mol L$^{-1}$ $Na_2CO_3$ was added into each spiked sample followed by 0.25 mL of a pH 7 phosphate buffer (prepared from $K_2HPO_4$ and $KH_2PO_4$), and 0.025 g $MgCl_2$. Magnetic stirrers were added into the centrifuge tubes and the mixtures were irradiated in a microwave at 95 °C for 1 h with a ramp time of 10 min.

In the extraction using alkaline EDTA solution, 0.2 g of a soil sample was weighed out into three polypropylene centrifuge tubes and spiked with $^{53}$Cr(VI) and $^{50}$Cr(EDTA)$^-$ standards. The $^{50}$Cr(EDTA)$^-$ standard was prepared by diluting the $^{50}$Cr(III) stock in 50.0 mmol L$^{-1}$ EDTA solution and heating the mixture in a microwave oven at 90 °C for 15 min. While $^{53}$Cr(VI) was spiked at expected analyte (Cr(VI))-to-spike ratio of 0.5–1.5, the amount of $^{50}$Cr(EDTA)$^-$ spike was kept at 5% of the total Cr in the corresponding sample. Then, 10.0 mL of 50.0 mmol L$^{-1}$ EDTA (pH 10) was added and magnetic stirrers were put into the tubes. The mixtures were irradiated in the microwave oven in two cycles, 90 °C for 5 min followed by 110 °C for 5 min with cooling to 25 °C between the cycles.[23]

After both extractions, the microwaved mixtures were cooled to ambient temperature and centrifuged at 4000 rpm for 20 minutes. The supernatants were carefully pipetted out into clean polypropylene tubes and stored at 4 °C until decomposition. The residues were preserved for analysis as described in the next section. Three method blanks were prepared for each extraction protocol.

### Decomposition of extraction residues for the determination of insoluble Cr

The residues from EPA Method 3060A extraction were transferred into microwave vessels and digested directly in a mixture of 9 mL $HNO_3$ and 0.5 mL HF following EPA Method 3052.[27] The residues from the EDTA extraction were transferred into microwave vessels, spiked with appropriate amounts of $^{50}$Cr(III) and decomposed in acid as described above. The digests were diluted and stored in a clean cold-room at 4 °C.

### Analysis of the digests and extracts

The digests generated from the soil samples and the extraction residues were analyzed by ICP-MS after 100-fold dilution with 1% $HNO_3$. The soil extracts were analyzed using the anion exchange IC-ICP-MS method described in the results and discussion part. Table 1 gives the optimum conditions for both analyses.

### Analyte quantification

Two mathematical approaches were used for analyte quantification. The total Cr in the soil samples and the insoluble Cr left in the extraction residues were quantified by IDMS using the data generated from the analysis of the corresponding digests by ICP-MS. SIDMS equations were used to calculate the concentrations of Cr(VI) and soluble Cr(III) with simultaneous correction for interconversions between the species using the data generated from the analysis of the extracts by IC-ICP-MS. Both the IDMS and SIDMS equations are described in EPA Method 6800.[12] In both calculations (IDMS and SIDMS), the isotopic abundance of $^{52}$Cr was used to monitor the endogenous Cr species.

All the analytical data from the ICP-MS and IC-ICP-MS analyses were corrected for mass bias as described in EPA Method 6800.[12] Mass bias correction factors were determined by analyzing a 50.0 ng g$^{-1}$ $^{Nat}$Cr standard (by ICP-MS), and a mixed standard containing 50.0 ng g$^{-1}$ $^{Nat}$Cr(III) and $^{Nat}$Cr(VI) (by IC-ICP-MS) in triplicate at the beginning, middle and end of each analysis sequence. Both standards were prepared in the corresponding method blanks.

## Results and discussion

### Chromatographic method for separating Cr(VI) and soluble Cr(III)

Direct separation of Cr(VI) and Cr(III) by chromatography is difficult because the species possess opposite charges in solutions. The two Cr species in the soil extracts were separated using an anion exchange IC-ICP-MS method after derivatizing Cr(III) to a negatively charged complex of EDTA, *i.e.* Cr(EDTA)$^-$.[23,30] A 2.0 mmol L$^{-1}$ EDTA solution (pH 10) was used as a mobile phase. For the EPA Method 3060A extracts, a 5.0 mL portion of the extract was mixed with equal volume of 50.0 mmol L$^{-1}$ EDTA, and the mixture was heated in a microwave at 90 °C for 20 minutes. The microwave heating was used to accelerate the complexation of Cr(III) with EDTA as the reaction is slow at room temperature,[31] The resulting solution was analyzed after 5-fold dilution with ultrapure water. A separate derivatization step was not required for the extracts generated using EDTA solution as the Cr(EDTA)$^-$ complex was formed during extraction. Hence, the extracts were analyzed directly after 10-fold dilution with ultrapure water.

Fig. 1 shows a chromatogram for the separation of the two Cr species in a soil extract generated using alkaline EDTA solution. The IC-ICP-MS method achieved baseline separation between Cr(VI) and Cr(EDTA)$^-$ with sharp and symmetrical peaks in less than 9 min. For the EPA Method 3060A extracts, retention time shift and slight change in peak shape were observed (Fig. 2) which might be due to the strong alkalinity (pH 12) and matrix composition (high concentrations of $Na_2CO_3$ and NaOH) of the extracts.

### Determination of total Cr in the soil samples

The mass fractions of total Cr in the soil samples were determined by IDMS after decomposing the samples (spiked with $^{53}$Cr) as described in the Experimental section. A mixture of 9.0 mL of $HNO_3$ and 0.5 mL of HF was found to be optimum for the decomposition of all the soil samples. The soil digests were analyzed by ICP-MS (after appropriate dilution), and ion counts (cps) were recorded at mass-to-charge ratios ($m/z$) of 52 and 53. IDMS calculations for total Cr quantification were performed using the mass bias corrected isotopic ratios of $^{53}$Cr/$^{52}$Cr. SRMs 2709a and 2711a, which had certified total Cr contents, were used for method validation. The measured total Cr in the two SRMs (122 ± 3 and 52.1 ± 2.1 μg g$^{-1}$, respectively) were in statistical agreement with their certified values (130 ± 9 and 52.3 ± 2.9 μg g$^{-1}$, respectively) at 95% CL. The other soil samples were found to have 54–680 μg g$^{-1}$ total Cr (Table 2, column 7), and the validity of the results was verified using mass balance as discussed later.

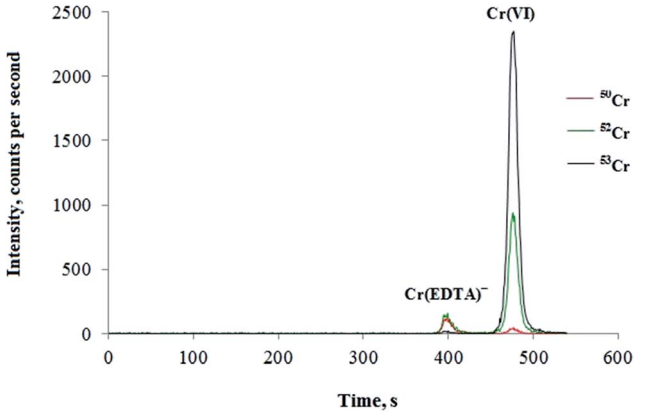


Fig. 1 Chromatogram showing the separation of Cr(III) and Cr(VI) in an extract generated from candidate SRM 2700g using alkaline solution of EDTA after double-spiking the soil with $^{53}$Cr(VI) and $^{50}$Cr(EDTA)$^-$.

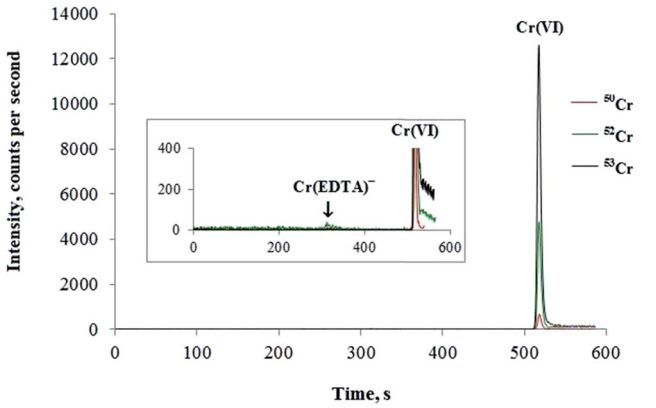


Fig. 2 Chromatogram for an extract generated from candidate SRM 2700q according to EPA Method 3060A after double-spiking the soil with $^{53}$Cr(VI) and $^{50}$Cr(III). The insert in the figure is a magnified section of the chromatogram showing the retention time of Cr(III) which was eluted as Cr(EDTA)$^-$.

### Determination of Cr(VI) in the soil samples

As explained in the previous sections, two extraction methods, *i.e.* EPA Method 3060A[22] and alkaline solution of EDTA,[23] in conjunction with double-spiking SIDMS were used to determine

 

the mass fractions of Cr(VI) in the soils (see the Experimental section for the procedures). The double-spiking strategy enables quantification of Cr(VI) and soluble Cr(III) in the extracts with simultaneous tracking and correcting for the interconversion between the two species.

### Extraction using EPA Method 3060A

Fig. 2 shows a chromatogram for the analysis of an extract generated from candidate SRM 2700q using EPA Method 3060A after spiking the sample with $^{53}$Cr(VI) and $^{50}$Cr(III). Similar chromatographic results were obtained for all the other soil samples. Absence of $^{52}$Cr(EDTA)$^-$ peak in the chromatogram indicates that no endogenous Cr(III) was solubilized from the soil, and similarly, absence of $^{50}$Cr(EDTA)$^-$ peak proves that the spiked $^{50}$Cr(III) did not stay in the alkaline extraction solution. The $^{52}$Cr(VI) peak in the chromatogram represents the endogenous Cr(VI) extracted from the soil and the Cr(VI) that came from method-induced oxidation of endogenous Cr(III), and the $^{53}$Cr(VI) peak represents the Cr(VI) spiked into the sample. The tiny $^{50}$Cr(VI) peak represents the spiked $^{50}$Cr(III) oxidized to $^{50}$Cr(VI) during or after extraction. Absence of $^{53}$Cr(EDTA)$^-$ peak in the chromatogram confirms that the spiked $^{53}$Cr(VI) was not reduced to form soluble Cr(III). These results were in good agreement with previous studies which reported that under the extraction condition of EPA Method 3060A, Cr(VI) dissolves from the soil into the extraction solution while Cr(III) falls out of solution and stays in solid phase.[15–19]

The Cr species in the extracts were determined by SIDMS with simultaneous tracking and correction for *in situ* transformation of species. In addition to the absence of method-induced reduction of the spiked $^{53}$Cr(VI) to soluble Cr(III) (see above), the $^{53}$Cr/$^{52}$Cr isotopic ratio in the residues of the soils left after EPA Method 3060A extraction (0.1175–0.1217) were found to be close to the natural ratio of the two isotopes (0.1134)[32] confirming that the spiked $^{53}$Cr(VI) did not precipitate as insoluble Cr. Therefore, the reduction of Cr(VI) to Cr(III) was disregarded and only the conversion of Cr(III) to Cr(VI) was considered and corrected for in the SIDMS calculations using the $^{50}$Cr(VI)/$^{52}$Cr(VI) and $^{53}$Cr(VI)/$^{52}$Cr(VI) isotopic ratios. Table 2 summarizes the mass fractions of endogenous Cr(VI) in the samples, after correction for the species transformations, and the percentages of Cr(III) that were converted to Cr(VI). The measured mass fractions of Cr(VI) in SQC012 ($53.8 \pm 0.8$ μg g$^{-1}$) and candidate CRM 2700q ($14.5 \pm 0.7$ μg g$^{-1}$) were in statistical agreement with their certified ($56 \pm 3.04$ μg g$^{-1}$)[28] and proposed certified ($14.9 \pm 1.2$ μg g$^{-1}$)[23] values, respectively, at 95% CL. The other soil samples were found to contain 0.8–13.6 μg g$^{-1}$ Cr(VI). In all the samples, very small percentage (<3%) of the endogenous Cr(III) was oxidized to Cr(VI) during and/or after extraction.

### Extraction using alkaline solution of EDTA

Alkaline solutions containing EDTA are demonstrated to be effective for extracting Cr(VI) along with soluble Cr(III) from soil *via* stabilizing the Cr(III) in the extract as Cr(EDTA)$^-$ complex.[23,33] In the present study, the soil samples were spiked with $^{53}$Cr(VI) and $^{50}$Cr(EDTA)$^-$ at the very beginning of the sample preparation step (see Experimental section). The spiking of Cr(III) as $^{50}$Cr(EDTA)$^-$ was to make sure that the spike stays in the alkaline extraction solution so that the mass fraction of solubilized endogenous Cr(III) can be determined with simultaneous tracking and correction for the method-induced interspecies transformation between Cr(VI) and the soluble Cr(III). As the soluble fraction of Cr(III) in soils is reported to be very small,[4,5,23] each soil sample was spiked with an amount of $^{50}$Cr(EDTA)$^-$ equivalent to 5% of its total Cr.

Fig. 1 shows a chromatogram for an extract generated from candidate SRM 2700g (similar chromatographic results were obtained for all the other soil samples). Unlike the results found using EPA Method 3060A, a $^{52}$Cr(EDTA)$^-$ peak was observed in the chromatogram which indicates the solubilization of a small fraction of endogenous Cr(III) from the soil. Furthermore, the $^{50}$Cr(EDTA)$^-$ peak shows the stabilization of the spiked Cr(III) in the extraction solution. The very small $^{53}$Cr(EDTA)$^-$ peak represents the method-induced reduction of a small fraction of the $^{53}$Cr(VI) spike into $^{53}$Cr(III). The $^{52}$Cr(VI) peak represents the endogenous Cr(VI) extracted from the soil, and the small $^{50}$Cr(VI) peak was of the Cr(VI) formed from the *in situ* oxidation of the spiked $^{50}$Cr(EDTA)$^-$. The $^{53}$Cr(VI) peak in the chromatogram represents the Cr(VI) spiked into the soil sample and remained in solution as chromate ion.

Since both the endogenous and the spiked Cr(VI) and Cr(III) were found in the soil extracts, the SIDMS calculations were carried out by correcting for both *in situ* species transformations, *i.e.* oxidation of Cr(III) to Cr(VI) and reduction of Cr(VI) to Cr(III), using the mass bias corrected $^{50}$Cr(III)/$^{52}$Cr(III), $^{53}$Cr(III)/$^{52}$Cr(III), $^{50}$Cr(VI)/$^{52}$Cr(VI) and $^{53}$Cr(VI)/$^{52}$Cr(VI) isotope ratios obtained from the chromatographic data. The mass fractions of Cr(VI) and soluble Cr(III) in the soil samples, after correction for the conversions between Cr(VI) and Cr(III), and the percentages of the interspecies transformations are summarized in Table 3. The mass fraction of Cr(VI) measured in SQC012 ($54.9 \pm 1.4$ μg g$^{-1}$) statistically agreed with the certified value ($56 \pm 3.04$ μg g$^{-1}$)[28] at 95% CL. For candidate CRM 2700q the experimental value of Cr(VI) ($13.5 \pm 0.4$ μg g$^{-1}$) was slightly less than the proposed certified mass fraction ($14.9 \pm 1.2$ μg g$^{-1}$).[23] For the other samples, the Cr(VI) mass fractions found using the EDTA extraction protocol (0.6–10.1 μg g$^{-1}$) were in good agreement with the values found using EPA Method 3060A (see Table 2). Table 3 also lists the fraction of soluble Cr(III) found in the soil extracts. For all the tested samples, less than 2% of the total Cr was solubilized into the alkaline EDTA solution to form Cr(EDTA)$^-$. The method-induced oxidation of Cr(III) to Cr(VI) in the EDTA extraction was very small (<2%) for all the samples. It has been demonstrated that the addition of EDTA into the extracting solution minimizes the oxidation of Cr(III) to Cr(VI).[23,33] The *in situ* reduction of Cr(VI) to Cr(III) was in the range 3–6%. Such reduction of Cr(VI) in alkaline EDTA solution was reported previously.[23,33] Components of soil including humic matter,[34] Fe(II) and sulfide[35,36] can reduce Cr(VI) to Cr(III) in mild alkaline conditions where they can be released from soil.[9,37]

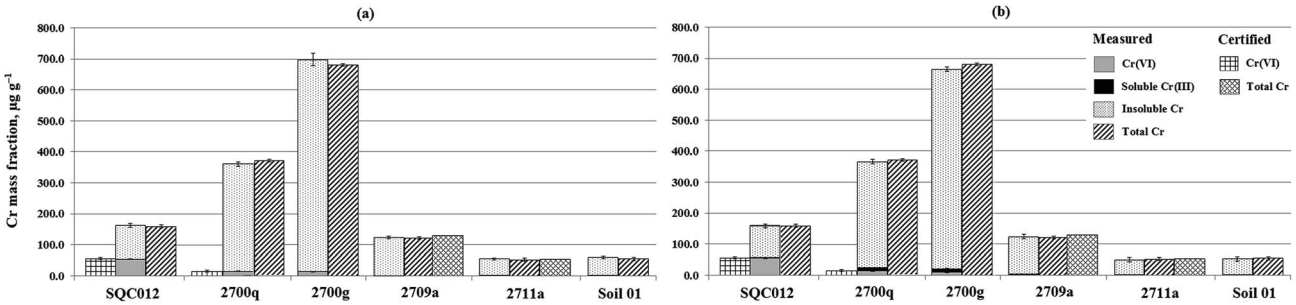


Fig. 3 Mass balance comparison between certified Cr mass fractions, total Cr and the sum of the fractions of Cr species in the soil samples ($n$ = 12, 95% CL). (a) EPA Methods 3060A and 3052 and (b) alkaline solution of EDTA and EPA Method 3052 were used for the determinations.



### Determination of insoluble Cr(III) in the soil samples

The insoluble Cr fractions in the soil samples, which mainly constitute Cr(III),[4,5] were determined by digesting the residues left after extraction following EPA Method 3052 (see Experimental section). The residues from EPA Method 3060A extraction were digested directly as the soils were initially spiked with $^{50}$Cr(III) and the spike quantitatively fell out into the residue (see the discussion above). The residues from the EDTA extraction were spiked with $^{50}$Cr(III) and digested. Unlike the strategy in EPA Method 3060A, in the EDTA extraction, the insoluble fraction of Cr cannot be quantified by spiking the soil with $^{50}$Cr(III) at the very beginning of the extraction step because the $^{50}$Cr(III) remains in the extraction solution forming $Cr(EDTA)^-$ complex and will not be equilibrated with the insoluble fraction of Cr in the soil.

The digests of the residues were analyzed by ICP-MS, and the insoluble Cr was determined by IDMS using the $^{50}$Cr/$^{52}$Cr isotopic ratio. Isobaric interference from $^{50}$Ti and $^{50}$V on $^{50}$Cr were corrected mathematically using the interference-free isotopes of Ti and V (*i.e.* $^{47}$Ti, $^{51}$V) as follows.[38] cps $^{50}$Cr = cps $m/z$ 50 − [(%$^{50}$Ti/%$^{47}$Ti) (cps $^{47}$Ti)] − [(%$^{50}$V/%$^{51}$V) (cps $^{51}$V)]; where %$^{47}$Ti, %$^{50}$Ti, %$^{50}$V and %$^{51}$V are the percent natural abundances of $^{47}$Ti, $^{50}$Ti, $^{50}$V and $^{51}$V, respectively. The IDMS calculations were made using the interference and mass bias corrected isotopic ratios of $^{50}$Cr/$^{52}$Cr. Tables 2 and 3 show the mass fractions of insoluble Cr determined in the residues left after EPA Method 3060A and EDTA extractions, respectively. The insoluble Cr left from the two extraction protocols accounted for 69–107% and 64–98% of the total Cr in the soil samples, respectively. In both cases, the lowest fraction of insoluble Cr was found in CRM SQC012 in which 34% of the Cr was Cr(VI). The validity of these results was evaluated using mass balance comparison as discussed in the following section.

### Mass balance

In addition to the use of certified reference materials, the validity of the analytical results was evaluated using mass balance by comparing the sum of the measured mass fractions of Cr(VI), soluble Cr(III) and insoluble Cr with the total Cr in the corresponding soil samples. For the results found by coupling EPA Methods 3060A and 3052, the mass balance comparison shows that the sum of Cr(VI) and insoluble Cr represented 96–109% of the total Cr measured in the samples (Table 2, last two columns). Similarly, comparison of the total Cr and the sum of the mass fractions of Cr(VI), soluble Cr(III) and insoluble Cr found using the EDTA extraction protocol integrated with EPA Method 3052 shows that mass balance was achieved for all the samples with the sum of the individual species representing 96–101% of the total Cr measured in the samples (Table 3, last two columns). Fig. 3 shows the comparisons between the experimentally measured Cr fractions with the certified values of Cr(VI) and total Cr in the reference materials.

## Conclusions

The study demonstrates the effective quantitation of hexavalent, soluble trivalent and insoluble chromium species in soil by coupling existing methods of Cr(VI) and total Cr determination with EPA Method 6800. Appropriate spiking strategies were designed to achieve equilibrium between the endogenous and spiked species while integrating the Cr(VI) extraction protocols, which use alkaline solutions of NaOH–$Na_2CO_3$ (EPA Method 3060A) and ethylenediaminetetraacetic acid (EDTA), with EPA Method 3052 to determine the Cr species sequentially. The use of the SIDMS methodology enables to track and correct for the transformation of species that may have occurred at the various sample-processing steps along with correction for partial analyte recovery, analyte loss, signal suppression and instrument drift. The proposed strategies were validated using several standard reference materials. Furthermore, mass balance was achieved between the sums of the measured fractions of Cr with the total content of the element for all the samples demonstrating the validity of the devised strategies for evaluating the distribution of Cr species in soil.

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